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The Effects of Different Strength of MS Media on *in Vitro* Propagation and Rooting of Spathiphyllum

Farklı Güçteki MS Besin Ortamlarının Spathiphyllum'un *in Vitro* Çoğaltımı ve Köklenmesi Üzerine Etkileri

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FARKLI GÜÇTEKI MS BESIN ORTAMLARININ SPATHIPHYLLUM'UN *IN VITRO* ÇOĞALTIMI VE KÖKLENMESI ÜZERINE ETKILERI

ÖZ:

Spathiphyllum Araceae familyasına ait bir iç mekan süs bitkisidir. Doğada 36 türü bulunan Spathiphyllum cinsinin doğada 30 türü bulunmaktadır. Spathiphyllum çok güçlü büyüyen ve bakımı kolay iç mekân bitkisidir. Spathiphyllum'un vejetatif çoğaltımının zor olduğu bilinmektedir. Bu nedenle mevcut üretim talebi yeterince karşılayamamaktadır. Artış gösteren spathiphyllum ihtiyacını karşılamak için klasik üretim yöntemleri ile bitki doku kültürü yöntemleri de kullanılmaktadır. Bu çalışmada, spathiphyllum'un mikroçoğaltımı ve köklenmesi üzerine MS besin ortamlarının farklı kuvvetlerinin (tam, ½ ve ¼) etkilerinin belirlenmesi amaçlanmıştır. Sürgün uçları, mikroçoğaltım için 1 mg L^{-1} BAP eklenmiş farklı kuvvetteki MS ortamlarında kültüre alınmıştır. Mikroçoğaltımdan gelen bitkicikler, köklenme için 1 mg L^{-1} IBA ilave edilmiş farklı güçteki MS ortamlarına aktarılmıştır. Köklü bitkiler kontrollü sera koşullarına alıştırılmıştır. Mikroçoğaltımda tüm parametrelerde ortamın en iyi gücü tam MS olarak tespit edilmiştir. Köklenme oranı her ortam için %100 olarak belirlenmiştir. Ancak kök sayısı, kök uzunluğu açısından ortamlar arasında önemli farklılıklar tespit edilmiştir.

Anahtar Kelimeler: Mikroçoğaltım, Bitki doku kültürü, BAP, IBA, Chico.

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THE EFFECTS OF DIFFERENT STRENGTH OF MS MEDIA ON *IN VITRO* PROPAGATION AND ROOTING OF SPATHIPHYLLUM

ABSTRACT

The Spathiphyllum is an indoor ornamental plant belonging to the Araceae family. Spathiphyllum genus, which has 36 species in nature, is a robust growth and easy to care for an indoor plant. Vegetative propagation of spathiphyllum is known to be complicated. For this reason, current production does not meet the demand sufficiently. Today, plant tissue culture methods and classical production methods are used to meet the increasing demand for spathiphyllum. In the present study, we aimed to detect the effects of different strengths (full, ½, and ¼) of MS media on micropropagation and rooting of spathiphyllum. Shoot tips of spathiphyllum 'chico' variety were cultured in different strengths of MS media containing 1 mg L^{-1} BAP for micropropagation. Plantlets obtained from micropropagation were transferred to different strengths of MS media containing 1 mg L^{-1} IBA for rooting. Plantlets in magenta boxes were removed and replaced in a controlled greenhouse. The best strength of medium was detected as the full MS in all parameters in vitro

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propagation. The rooting rate was determined 100% for each medium. However, there are significant differences among the media in view of the number of roots and length of roots.

Keywords: Micropropagation, Plant tissue culture, BAP, IBA, Chico.

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1. INTRODUCTION

Türkiye has significant advantages in cultivating ornamental plants due to its favorable climatic and geographical conditions, proximity to market countries and cheap labor. Ornamental plants have been used aesthetically and functionally for centuries. Today, ornamental plants play an active role in eliminating the longing for nature, which is one of the adverse effects of urbanization and making urban environments more livable. Ornamental plants constitute a general concept and consist of four different groups according to their use. These groups are cut flowers, outdoor ornamental plants, seasonal flowers, indoor ornamental plants and bulbous and tuberous plants. The wide usage area of ornamental plants and their preference in different environments and areas positively affect the product range. Especially considering the effects of plants on nature-human relationships, it is possible to perceive the functionality of plants in health dimensions. Since indoor ornamental plants are also included in the living environments of individuals, personal preferences can affect the way they are used. Indoor ornamental plants are known for decorative leaves, flowers, or stems suitable for growing in pots, containers, or crates. In other words, they are plants that can be grown indoors (home, office, etc.) or semi-closed (balcony). Indoor ornamental plants increase air quality and strengthen the bond between humans and nature. 47% of the world population (about 2.9 billion people) lived in urban areas between the years 2000, and it is estimated that this rate will increase to 60% in 2030. Considering that 80% of the people living in big cities live indoors, this is a fact that affects human health. Indoor plants have positive effects on patient health in symptoms such as psychophysiological stress due to laboratory experiments and quasi-experimental field studies. (Bringslimark et al., 2009; Richardson et al., 2013; Yazici, 2020). The origin of Spathiphyllum, an indoor plant belonging to the Araceae family, is Colombia and Venezuela. Spathiphyllum genus, which has 36 species in nature, is a robust growth and easy for care houseplant plant. The spathiphyllum leaves consist of green, long oval, pointed flowers towards the tip, white, spathe and spadix. The spadix that surrounds the flower head is also called "Barış Zambağı" because it is white or "Beyaz Yelken" because of its similarity to sail in the local Turkish market. Vegetative propagation of spathiphyllum is complex because the annual producing coefficient and the survival rate are low (Fan et al., 2019). This situation limits commercial production and leads to the inability to meet the demand for spathiphyllum. To-

day, biotechnological methods and classical production methods are used to meet the increasing demand for spathiphyllum. In plant breeding and cultivation, many different biotechnological methods could be applied to plants. One of the broadest branches of plant biotechnology is plant tissue culture (Dönmez, 2022). Today, micropropagation, organogenesis, somatic embryogenesis, somatic hybridization, haploid plant production, virus elimination, production of secondary metabolites are routinely used as different plant tissue culture techniques (Donmez et al., 2016). Due to the increase in the popularity of the spathiphyllum plant in recent years, manufacturers are using plant tissue culture techniques to meet the demand needed in the market. Micropropagation is one of the plant tissue culture applications that allows clonal and rapid propagation of plants in aseptic conditions (Sevindik et al., 2017). Micropropagation, which is included in plant tissue culture techniques, is being used more and more for rapid clonal propagation of various economic plants and the protection of germplasm. In addition, micropropagation is a powerful tool for the reproduction of valuable materials obtained after hybridization studies (Şimsek et al., 2018; Kurtuluş et al., 2021). Media composition is one of the most critical factors influencing plant growth (Rezali et al., 2017; Tütüncü et al., 2019).

Success in micropropagation and *in vitro* rooting studies are affected by the plant tissue culture media content depending on plant species. Therefore, different culture media can be used in *in vitro* (Şimşek et al., 2017). MS (Murashige and Skoog, 1962) nutrient medium is one of the most widely used nutrient media in plant tissue culture studies. Reducing the cost of nutrient media used in plant tissue culture studies is commercially very important. Until now, different researchers have carried out micropropagation studies in different varieties of spathiphyllum (Das et al., 2000; Ramirez-Malagon et al., 2001; Dewir et al., 2005; Teixeira da Silva et al., 2006; Bandyopadhyay et al., 2011; Ibrahim et al., 2016; Kaçar et al., 2020), but there is not any study on the different strengths of the MS medium for spathiphyllum in micropropagation studies. In the present study, we aimed to detect the effects of different strengths (full, ½, and ¼) of MS media on *in vitro* propagation and rooting of spathiphyllum

2. MATERIALS AND METHODS

2.1. Plant Material and Surface Sterilization

Spathiphyllum 'Chico' genotype was used as plant material. Firstly, shoot tips of spathiphyllum 'Chico' were washed for 10 minutes under tap water. Then the shoot tips were kept in 15% sodium hypochlorite + 1-2 drops of Tween 20 solution for 20 minutes. Then, shoot tips were washed three times with sterile distilled water to remove sterilant agents.

2.2. Preparation of MS Media

Different strengths (full, ½ and ¼) of MS media were used for the *in vitro* propagation and rooting of spathiphyllum 'Chico'. The culture medium comprises of 4.4 g L^{-1} MS media for full strength medium, 2.2 g L^{-1} for ½ strength MS medium, 1.1 g L^{-1} for ¼ strength MS medium (Duchefa Biochemie, Netherland), 30 g L^{-1} of sucrose and 7 g L^{-1} agar (Duchefa Biochemie, Netherland). The medium pH was adjusted to 5.7–5.8. Then the medium was autoclaved for 15 min at 121 °C and 1.05 kg cm-2.

2.3. *In vitro* propagation and rooting

Shoot tips were cultured in different strengths (full, ½ and ¼) of MS medium added 1 mg L^{-1} BAP for micropropagation. The plantlets were subcultured 3 times in total, once every four weeks in different strengths (full, ½ and ¼) of MS media. At the end of each subculture, the number of leaves, plant length (cm), micropropagation rate, fresh weight (g) were recorded. *In vitro* rooting experiments were established with the plants obtained as a result of micropropagation. Plantlets from micropropagation were transferred to different strengths (full, ½ and ¼) of MS media supplemented with 1 mg L^{-1} IBA for rooting. All plants were cultured in a growth chamber at 25±2 °C under cool white fluorescent light at 16 h photoperiod conditions. After six weeks, numbers of roots, rooting rate (%), length of roots (cm) and length of plants (cm), fresh weight (g) were recorded.

2.4. Acclimatization

Roots of plants were kindly washed with running tap water, and 50% (w/v) of a 2.5 g L^{-1} fungicide was performed with immersing for 15-20 s. Then, plants were transferred to plastic pots containing autoclaved peat and perlite (1:1, v/v). Afterward, spathiphyllum 'Chico' plants were moved to a controlled greenhouse under natural light at 22-24 °C and 95-98% relative humidity.

2.5. Data analysis

The study was carried out according to a completely randomized design. Studies were performed with 3 repetitions, 10 plants in each replication. Micropropagation rate, rooting rate (%), numbers of roots, length of roots (cm), length of plants (cm), fresh weight (g) were calculated, and variance analysis was carried out. Means defined as statistically significant were separated by LSD (least significant difference test) to measure differences among different strength (full, ½ and ¼) of MS in spathiphyllum 'Chico'. JMP® software was used for statistical analysis.

3. RESULTS AND DISCUSSION

3.1. *In vitro* propagation

Data of the number of leaves, plant length (cm), micropropagation rate, fresh weight (g) coming from different strengths (full, ½ and ¼) of MS media were presented in Table 1.

Table 1. Data of *in vitro* propagation treatments in different strengths (full, ½ and ¼) of MS media for spathiphyllum

Strength of Medium	Micropropagation Rate	Plant Length (cm)	Number of Leaves	Fresh Weight (g)
Full MS	8.78a	3.64a	23.95a	2.31a
½ MS	4.40b	2.22b	10.33b	1.35b
¼ MS	3.68b	1.44c	7.41c	0.91c

LSDMicropropagation rate=0.93, LSDPlant length=0.26, LSDNumber of leaves=2.47, LSDFresh weight=0.35

The best strength of medium was detected as the full MS in all parameters recorded in *in vitro* propagation. The micropropagation rate of spathiphyllum in the full MS was determined as 8.78. However, it was determined as 4.40 and 3,68 in ½ MS and ¼ MS media, respectively. Maximum plant height was determined in plants growing in full MS medium with an average of 3,64 cm. Similarly, the greatest number of leaves was determined in plants cultured in full MS medium with an average of 23.95. However, this number decreased to 7.41 in ¼ MS media. Plants growing in the different strengths (full, ½ and ¼) of MS media containing 1 mg L^{-1} BAP were presented in Figure 1.

Figure 1. Plants growing in the different strengths (full, ½ and ¼) of MS media containing 1 mg L^{-1} BAP

3.2. *In Vitro* Rooting

Data of numbers of roots, length of roots (cm) and length plants (cm) were presented in Table 2.

Table 2. Data of *in vitro* rooting treatments in different strengths (full, ½ and ¼) of MS media for spathiphyllum

Strength of Medium	Numbers of Roots	Length of Roots	Length of Plant	Number of Leaves
Full MS	14.00c	4.15b	2.29b	7.50a
½ MS	19.03b	4.72ab	2.57a	7.15a
¼ MS	21.42a	5.77a	2.42ab	6.60b

$LSD_{Numbers\ of\ roots}$=1.94, $LSD_{Length\ of\ roots}$=1.44, $LSD_{Length\ of\ plant}$ =0.18, $LSD_{Number\ of\ leaves}$=0.46

All plants cultured in different strengths (full, ½ and ¼) of MS media containing 1 mg L^{-1} IBA were rooted. The rooting rate was determined 100% for each medium. However, there are significant differences among the media in view of the number of roots and length of roots. The best medium was detected as ¼ MS for the number of roots (21.42) and length of roots (5.77 cm). As the strength of the MS media decreases, the quality of root development increases. Plants growing in the different strengths (full, ½ and ¼) of MS media containing 1 mg L^{-1} IBA were presented in Figure 2.

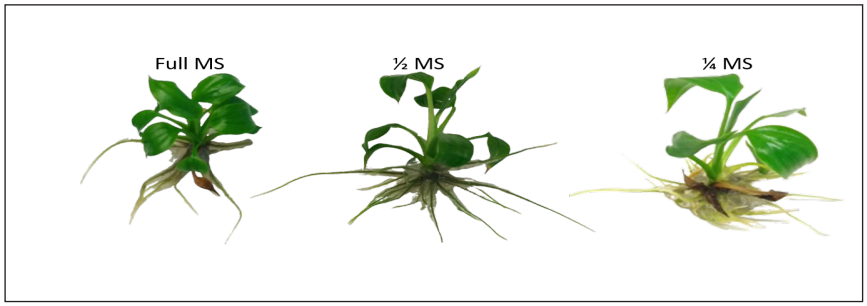

Figure 2. Plants growing in the different strengths (full, ½ and ¼) of MS media containing 1 mg L^{-1} IBA

3.3. *In Vitro* Rooting

Rooted plants from different strengths (full, ½ and ¼) of MS media were moved to plastic pots. The plants were successfully survived in the greenhouse conditions. There were no statistical differences among the treatments and the survival rate were %100 in all treatment. Plant growing in the greenhouse are presented in Figure 3.

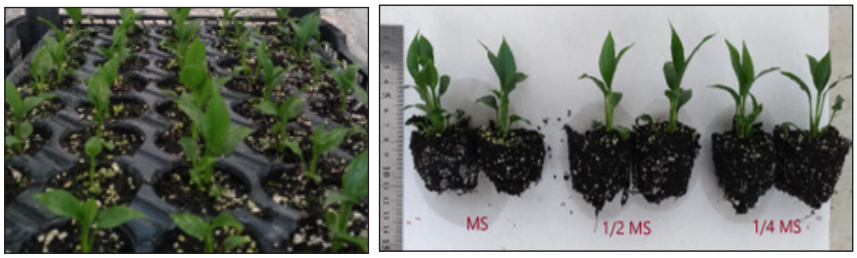

Figure 3. Plant growing in the greenhouse.

The content of the medium, type and concentration of plant growth regulator are very important in plant tissue culture experiments. Ramires-Malagon et al (2001) reported that a maximum of 11.6 shoots per explant were obtained from *in vitro* micropropagation of cultivar Petite (*Spathiphyllum floribundum* cv. Petite). Dewir et al. (2006) used different concentrations of IBA and NAA together with BA in their micropropagation studies conducted on *S. cannifolium* to determine the most effective protocol. They obtained the highest number of shoots in the medium containing 13.32 mM BA and 4.9 mM IBA. Kaçar et al. (2005) used MS medium containing BA and PBA plant growth regulators at different concentrations for the micropropagation of 'Sweet Pablo' spathiphyllum variety and stated that PBA gave more effective results than BA in obtaining healthy and strong shoots.

Geneyikli (2009) used different concentrations of Kin, BA and NAA to determine the most successful plant growth regulator on micropropagation of three different spathiphyllum cultivars (Sweet Dario, Sweet Chico, Sweet Cupido) and found that the most effective micropropagation medium for all three cultivars was MS medium containing 1 mg L^{-1} BA. Ibrahim et al. (2016), reported that MS medium supplemented with 0.5 mg L^{-1} BA gave the maximum shoots numbers/explant in *S. connifolium*.

The MS medium generally gives successful results in spathiphyllum micropropagation studies. However, there is not any study on the different strengths of the MS medium for spathiphyllum. In some plant tissue culture studies, it was determined that higher success was achieved at low strengths of MS. Lee and Paek (2012), compared the different strengths of MS (2, 1, 3/4, 1/2 and 1) to produce biomass and bioactive compounds of *Eleutherococcus koreanum* Nakai, growing in forests of Jeju Island. They reported that 1/2 MS is the best suitable strength for bioactive compound and biomass productions. Hilae and Te-chato (2005) reported that they obtained better results by reducing the strength of MS for root formation in oil palm somatic embryo germination. In plant tissue culture, both private organizations and research institutions give importance to the cost issue. With this study, it has been shown that savings can be achieved by using low concentrations of MS. It has been proven that similarly successful results are obtained with lower costs.

4. CONCLUSION

The demand for spathiphyllum, which has an important place in the ornamental plant market, is increasing day by day. For this reason, it is important to benefit from classical methods and tissue culture techniques in spathiphyllum propagation. It is important to reduce the cost of plant tissue culture studies. In this study, the effects of using lower concentrations of MS on spathiphyllum micropropagation and rooting were investigated. We attempted to obtain a cost-effective *in vitro* micropropagation and rooting protocol for spathiphyllum. For this purpose, different strengths (1, 1/2 and 1/4) of MS were used in the study. The best results in micropropagation were obtained from 1 MS strength. In the rooting stage, no difference was found among the strengths of MS. In addition, acclimated plants showed good growth even though they came from low MS strengths. These results showed that 1/2 and 1/4 MS strengths could be used instead of full MS in *in vitro* rooting studies of spathiphyllum.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethics

This study does not require ethics committee approval.

Author Contribution Rates

Design of Study: DD (%50), MHE (%10), BB (%10), ÖŞ (%15), YAK (%15)

Data Acquisition: DD (%50), MHE (%15), BB (%10), ÖŞ (%15), YAK (%10)

Data Analysis: DD (%50), MHE (%5), BB (%5), ÖŞ (%20), YAK (%20)

Writing Up: DD (%50), MHE (%5), BB (%5), ÖŞ (%20), YAK (%20)

Submission and Revision: DD (%50), MHE (%0), BB (%0), ÖŞ (%0), YAK (%50)

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